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THE EFFECT OF DENERVATION ON THE MECHANICAL AND ELECTRICAL RESPONSES OF FAST AND SLOW MAMMALIAN TWITCH MUSCLE

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SUMMARY

1. The soleus (slow twitch), flexors digitorum and hallucis longus muscles (fast twitch) of the cat were denervated. Isometric contractions and electrical responses were examined 2–160 days after the operation.

2. In the first week the time course of the twitch and the ratio of tetanus tension to twitch tension were normal in both muscles. The maximum rate of rise of tension in the tetanus was reduced in fast muscles.

3. In the second week all the twitches showed a normal contraction phase but relaxations were interrupted by a repetitive after-contraction. This became less marked after longer periods of denervation.

4. During the third and subsequent weeks, the contraction and relaxation phases of the twitches in all muscles became slower than normal. These changes were greater in fast muscles which, nevertheless, remained quite distinct from soleus. The ratio of tetanus tension to twitch tension fell below normal. It is suggested that these changes are brought about by more complete activation of the contractile proteins in a twitch. In flexor hallucis longus the rate of rise of tension in isometric tetani was found to be further reduced. No change was found in soleus.

5. In extracellular and intracellular records the initial response was a single action potential. An after-discharge occurred in a proportion of fibres during the relaxation phase of the twitch.

6. The intracellularly recorded action potential was smaller and had a longer duration than that of normal muscle. Refractory period increased. Conduction velocity decreased. These changes were greater in fast muscle and differences between fast and slow twitch muscle were less marked than

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in normal muscle. Fibrillation potentials occurred at the same rate in fast and slow muscle.

7. The mechanisms of the mechanical changes are discussed and the possible relevance to the question of motor nerve influence on muscle is indicated.

INTRODUCTION

Early observations of mammalian muscle following denervation (Langley, 1915; Denny-Brown, 1929; De Smedt, 1949) have shown that the contractions become slow but there was little detailed information or comparison between different muscle types. Investigations of isometric twitches of denervated mammalian fast-twitch and slow-twitch muscles have been reported (Eccles, Eccles & Kozak, 1962; Lewis, 1962) but although the authors found that fast muscle becomes slow there was disagreement about the changes in the slow muscle. The present results show in more detail the changes that occur in the twitch following denervation and the interpretation of these results is assisted by observations on isometric tetani. It is known that denervated muscle undergoes repetitive activity (Rosenblueth & Luco, 1937; Eccles, 1941) and this has been assessed by extracellular and intracellular techniques in the present experiments in order to test if the twitch response of denervated muscle is the result of a single propagated muscle action potential and therefore comparable to normal muscle.

The mechanisms of changes are discussed and a comparison is made with cross-innervated fast-twitch muscle (Buller, Eccles & Eccles, 1960). Only limited conclusions can be drawn on the more general problem of the influence that a motor nerve has on its muscle since the changes occurring in denervation appear to be substantially different from those following cross-innervation.

The intracellular recording was done in collaboration with Dr R.M.A.P. Ridge.

METHODS

Adult cats of between 1·5 and 4·5 kg body weight were anaesthetized with pentobarbitone sodium (40 mg/kg) injected intraperitoneally. The nerves to soleus and F.D.L. or F.H.L. were cut close to the muscles and the proximal stumps were cleared, pulled through lateral gastrocnemius and fixed on to its superficial fascia in order to reduce re-innervation. When the final experiment was to be performed within 1 week of the initial operation, denervation was made by section of the medial popliteal nerve to avoid muscle damage. Aseptic techniques were used and an injection of penicillin and streptomycin was given post-operatively.

The final experiments were performed 2–147 days after the operation of nerve section. The animals were induced and maintained with pentobarbitone. In preparing the muscles for myography care was taken to test for re-innervation before extensive dissection. The techniques used were similar to those described in detail

by Buller & Lewis (1965*a*, *b*). The dissected muscles were immersed in mineral oil maintained near 37° C. The tibia and fibula were transfixed at either end with steel twist drills which were fixed to a metal table but insulated from it electrically. The muscles were stimulated directly using the steel twist drill in the upper tibia as one electrode and a fine stainless-steel wire wound around the muscle tendon as the other. The tendon was covered with a slip of saline-soaked cotton-wool to prevent drying. The muscles were freed as far as possible without damaging their blood supply and often a thin plastic sheet was used to insulate the muscle from surrounding tissue. Isometric tension was recorded with unbonded wire strain gauges mounted on a micrometer which allowed the muscle to be extended. Myograms were recorded on film or analysed automatically (Buller & Lewis, 1965*b*).

It was necessary to define an adequate stimulus for these muscles and preliminary experiments were performed with normally innervated muscle paralysed by D-tubocurarine. Contractions equal to the original twitch tension could be evoked with pulses of more than 3 msec but these had times to peak greater than normal and were considered to be brief tetani. Pulses of 1 msec or less always produced submaximal contractions with normal or slightly short times to peak (cf. Lewis & Rosendorff, 1965). Similar results were obtained in denervated muscle, except that pulses of up to 3 msec could be used without distortion of the twitch probably because the refractory period increased after denervation (Fig. 3). In the majority of experiments stimuli of 0·1, 0·3 and 1·0 msec were used at 90 V, and a mean value taken from the resulting responses. It appeared to be impossible to stimulate the whole muscle without spread to adjacent muscles. After these measurements, denervated muscles and the corresponding muscles of the opposite limb were removed as close as possible to the bone, blotted and weighed, after removing the tendon at the insertion of the most distal muscle fibres. Over-all length was measured after the muscle had been pulled straight and then released.

Electrical activity was recorded in muscles either 1 week or 5–7 weeks after denervation. Extracellular potentials were recorded by Sherringtonian or concentric needle electrodes and amplified by a conventional preamplifier with 3 db points set at 80 Hz and 10 kHz. A stimulus isolating transformer and Wagnerian earth were used to minimize artifact and in some experiments a double cathode follower was placed between the electrodes and preamplifier. Despite this the amplifier often became blocked so that in later experiments stimulation was confined to a bundle of muscle fibres on the deep surface of the muscle, where connective tissue was minimal. These were held straight by a clip on their tendon or aponeurosis and a concentric needle electrode, ground flat at the tip, was used to stimulate the bundle at its origin. The stimulus was derived from an isolated stimulator. A flattened bipolar needle electrode was used to record the electrical response. This was held in a universal joint and a calibrated manipulator which permitted the needle tip to be moved in a line parallel to the muscle surface for measured distances. This technique was used to study the response following a single stimulus and also to measure conduction velocity.

A similar arrangement was used when recording intracellularly with microelectrodes. These were micropipettes filled with 3 M-KCl and having resistances in the range 15–20 MΩ. A system of capacity compensation was used with constant monitoring to achieve a time constant of less than 50 μsec. Movement was limited by stimulating a small bundle of fibres. Thus electrode wastage was minimal and many of the fibres could be recorded, without damage, following two or three stimuli. The techniques were similar to those used in normal mammalian muscle by Buller, Lewis & Ridge (1966).

RESULTS

The weights of the muscles fell rapidly over the first month, thereafter atrophy was slower. Soleus muscles between the fourth and ninth weeks of denervation had a weight of 58% of those of the opposite limb. Denervated F.H.L. was 41% and F.D.L. 57% of the control muscles. In the same period there was no decrease of the over-all length of the denervated muscles.

Chronaxies were measured from strength–duration curves. Normal soleus gave a mean value of 1·58 msec (S.E. of mean = 0·12, n = 12), and muscle denervated for more than 20 days 3·1 msec (S.E. of mean = 0·4, n = 11). The comparable figures for F.D.L. were 1·26 msec (S.E. of mean = 0·19, n = 13) and 3·2 msec (S.E. of mean = 0·3, n = 8).

Changes in the isometric twitch

Four typical isometric myograms are illustrated in Fig. 1 each accompanied by an analysis of the twitch (Buller & Lewis, 1965*b*). Each dot lasts 1 msec, the second (raised) group measures the time to peak tension

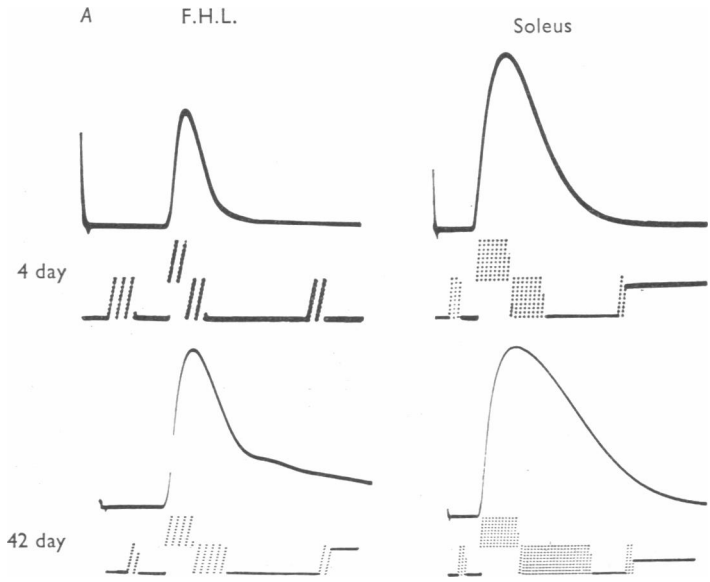

Fig. 1*A*. For legend see opposite page.

and the third the time from peak to half peak tension. The first and last groups indicate the passive and active tension respectively and the value of each dot is indicated in the legend. The records on the left are from F.H.L., those on the right from soleus. The upper pair are muscles de-

nervated for 4 days in which the contraction and relaxation times were within the normal range. The lower records are from an animal operated 42 days earlier, and it is seen that the times to peak and to half relaxation were increased in both F.H.L. and soleus.

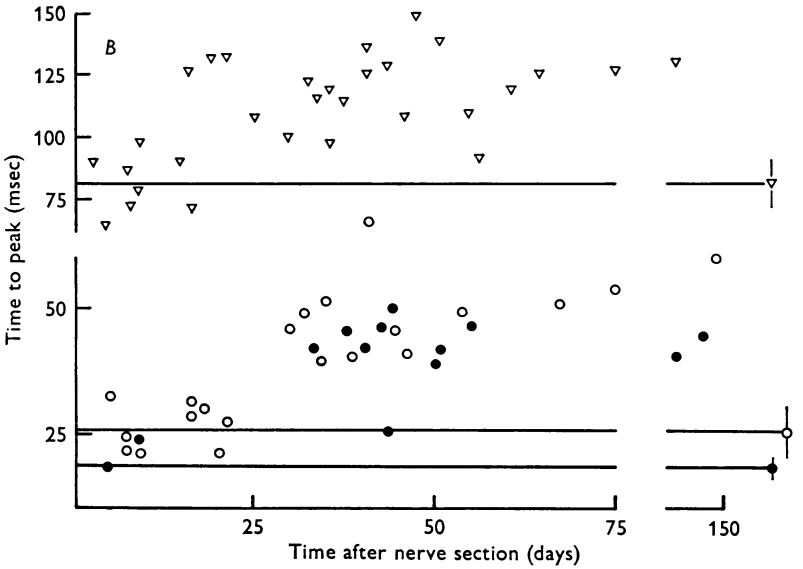

Fig. 1*A*, myograms of isometric twitches of muscles denervated for 4 days (above) and 42 days (below). On the left are records from F.H.L. and on the right from soleus. Below each trace is a digital representation of certain twitch parameters. The dots last 1 msec and are arranged in four groups. The first group indicates the initial tension on the muscle. The second, raised, group starts at the onset of contraction and ends at the time of peak tension and measures time to peak in msec. The third group measures from the peak to the time when the active tension fell to half its maximum value (time to half relaxation msec). The final group represents the peak active tension, each dot corresponding to 10 g for the 42 day F.H.L. record and 5 g for all others. *B*, graph of times to peak of soleus (▽), F.H.L. (●) and F.D.L. (○) muscles plotted against period of denervation. Note the discontinuity of the ordinate scale used for slow and fast muscles. The symbols on the far right and the horizontal lines show the mean values for control muscles. The vertical lines indicate ± 1 S.D.

The times to peak of twitches of muscles denervated for various periods are plotted in Fig. 1*B* as triangles for soleus, open circles F.D.L. and filled circles F.H.L. Different ordinate scales have been used for fast and slow muscle. The horizontal lines and symbols on the far right indicate means and the vertical bars show ± S.D. for control muscles. Muscles denervated for less than 3 weeks had times to peak within the normal range, those denervated for a longer period had a prolonged time to peak.

This change occurred consistently in fast and slow muscle, perhaps beginning a few days earlier in soleus. In this paper the term 'acutely denervated' will be used to describe muscles having a normal time to peak whilst those with a time to peak outside the normal range will be described as 'chronically denervated'. The means and S.E. for these groups of muscles are set out in Table 1. The similarity between acutely denervated muscles

TABLE 1. Contraction characteristics of control, acutely denervated and chronically denervated muscles. Mean values are given and, in brackets, the S.E. of the mean and the number of muscles. Control values indicated by * are quoted from Buller & Lewis (1965*a*)

		Denervated	
	Control	Acutely	Chronically
Time to peak twitch (msec)			
Soleus	79·4 (2·1, 28)	82·0 (3·6, 9)	120·8 (3·0, 23)
F.D.L.	27·4 (1·5, 16)	26·0 (1·7, 9)	49·9 (2·7, 10)
F.H.L.	18·3 (0·1, 11)	19·0 (1·0, 3)	41·8 (2·7, 8)
Time to half relaxation (msec)			
Soleus	92·0 (4·5, 28)	102·5 (7·1, 8)	218 (10·0, 18)
F.D.L.	24·7 (1·9, 16)	26·9 (3·1, 8)	78·5 (9·1, 11)
F.H.L.	15·7 (0·9, 11)	18·7 (3·2, 3)	53·4 (2·5, 10)
Tetanus: twitch ratio (msec)			
Soleus	4·42 (0·13, 11)	4·91 (0·24, 6)	2·82 (0·15, 17)
F.D.L.	4·71 (0·57, 6)	4·45 (1·15, 4)	2·24 (0·38, 5)
F.H.L.	4·15 (0·25, 11)	4·71 (0·62, 3)	2·36 (0·20, 7)
Maximum rate of rise (% P_0/msec)			
Soleus	1·53 (0·06, 10)*	1·40 (0·10, 7)	1·53 (0·04, 10)
F.H.L.	4·64 (0·24, 9)*	4·09 (0·35, 3)	3·09 (0·22, 7)
Optimum rate (Hz)			
Soleus	315*	200	195
F.H.L.	610*	460	230
Conduction velocity (m/sec)			
Soleus	2·95 (0·12, 4)	—	2·28 (0·09, 4)
F.H.L.	3·66 (0·17, 4)	2·90 (—, 1)	1·86 (0·11, 4)

and the controls is confirmed. The differences between the times to peak of chronically denervated muscle and controls are significant at the 0·001 level for the two fast and at 0·02 for the slow muscles. The ratios of the mean time to peak of chronically denervated muscle to that of the control were 1·51 (soleus), 1·82 (F.D.L.) and 2·29 (F.H.L.). In this late stage of denervation the twitches of fast muscle remained significantly faster than those of slow muscle.

There was even greater prolongation of the relaxation phase as shown in Fig. 2*B* in which the time to half relaxation is plotted against the period of denervation. The acutely denervated muscles are seen to have near-

normal half relaxation times, the mean ratios of the values of the operated to those of the control muscles was 1·09 for soleus (triangles) and F.D.L. (open circles) and 1·20 for F.H.L. (filled circles), none of these was significantly greater than 1. The comparable ratios of chronically denervated muscle were 2·61 (soleus), 3·01 (F.D.L.) and 3·36 (F.H.L.).

Measurements of the relaxation phase were complicated by the presence of a late repetitive contraction that was first seen 1 week after denervation. This after-contraction became most marked at about 2 weeks when it sometimes took the form of a contracture lasting seconds or even minutes. More commonly it resembled a short unfused tetanus of diminishing size. Typical examples are seen in Fig. 1*A* but some extreme cases are presented in Fig. 2*A*. It declined progressively in chronically denervated muscle. This time course may be contrasted with that of spontaneous fibrillation which appeared 3 days after denervation.

Over-all 39% of soleus, 46% of F.D.L. and 66% of F.H.L. muscles showed repetitive contraction. The time of onset of the after-contraction depended on the muscle and period of denervation. In acutely denervated soleus the mean time of onset was 121 msec after the stimulus, for F.D.L. the mean was 84 msec and for F.H.L. 95 msec. The corresponding figures for chronically denervated muscles were 183, 129 and 110 msec. Thus the after contraction often began before the tension had fallen to half the maximum in soleus and such muscles are indicated by the filled triangles in Fig. 2*B* (placed above the ordinate scale).

Post-tetanic effects. The after-contraction was very sensitive to previous activity. Two examples are shown in Fig. 2*A*. The upper left myogram was obtained in an acutely denervated F.H.L. which had been unstimulated for 15 min. After stimulation once every 6 sec the after-contraction was reduced (lower left). The after-contraction of one F.D.L. muscle was more resistant but a 500 msec tetanus at 100 Hz almost completely eliminated it (middle pair of records). The same phenomenon was seen in soleus muscles (right-hand pair) and in chronically denervated muscles.

In contrast to the effects of a tetanus on the after-contraction, the initial twitch was little affected. Post-tetanic potentiation was reduced in the second week of denervation and after that time fast muscles showed a depression of the same order as denervated and normal soleus. Very prolonged tetani (5 sec) decreased the time to peak of both fast and slow muscle twitches but never by more than 10%. Effects of this order are found in normal muscles. Three exceptions to this have been seen and one illustrated in Fig. 2*A* (left) has been discussed. Two other muscles in the second and third weeks of denervation were observed after a period of rest to have twitches with a prolonged time to peak which was reduced to normal by short tetanus.

Effect of muscle length. Active twitch (and tetanic) tensions of acutely and chronically denervated muscles were influenced by muscle length and showed a clear optimum. F.H.L. was twice as sensitive as soleus to changes of muscle length; a result similar to that found in normal muscle (Buller & Lewis, 1963). All time measurements presented above have been made at optimum length.

Refractory period. An estimate of the refractory period was made by measuring the mechanical response to two equal stimuli. In Fig. 3 peak tension (circles) and time to peak (triangles) have been plotted against

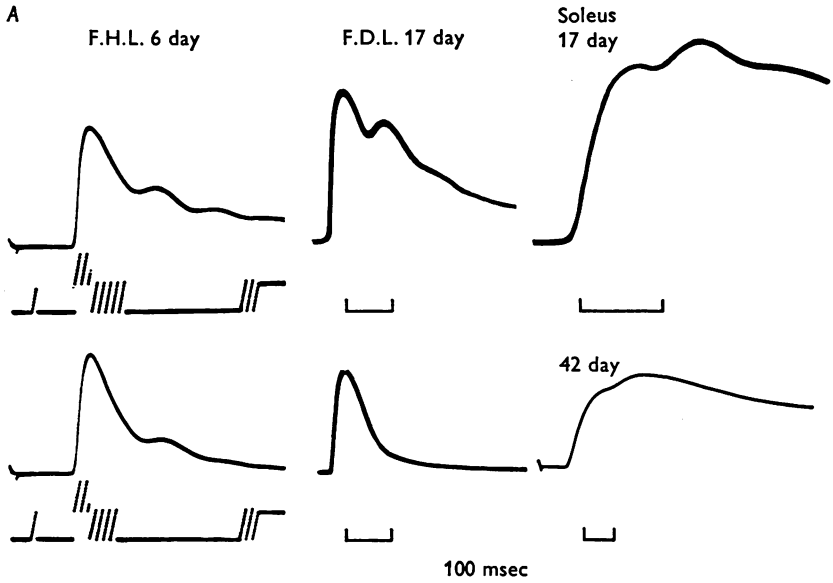

Fig. 2*A*. For legend see opposite page.

stimulus interval for muscles denervated 4 days (open symbols) and 56 days (filled symbols) for soleus (left) and F.H.L. (right). The stimuli were submaximal, and at the shortest interval summed to give a larger contraction (with no change in time to peak). As the interval was increased, the contraction initially became smaller until the tension began to increase again, now with a longer time to peak. This was taken to be a measure of the refractory period. In the muscle denervated 4 days mean values of 1·3 msec (F.H.L.) and 2·1 (soleus) were obtained and these are close to values obtained in single fibres of normal muscles (A. J. Buller, D. M. Lewis & R. M. A. P. Ridge, unpublished observations). In muscles denervated more than 20 days a higher value of 2·7 msec (S.E. of mean = 0·11 msec) was obtained in both F.H.L. (n = 4) and soleus (n = 5). A few observations using the extracellular or intracellular action potential

gave similar values. Much shorter values are obtained in normal muscles with indirect stimulation, but the end-plate potential has an amplitude well above threshold in mammalian muscle.

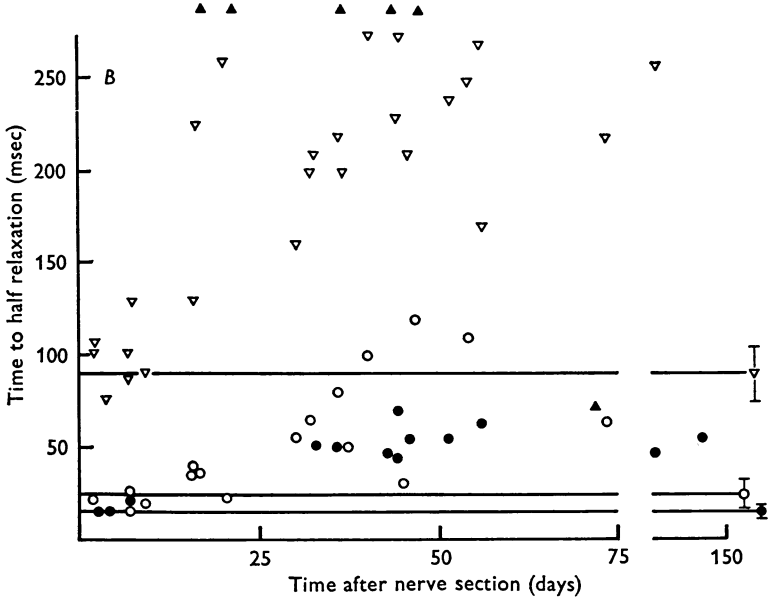

Fig. 2*A*, isometric twitch myograms of denervated muscles showing a marked after-contraction and the effect of muscle activity. The digital displays on the left have the same significance as in Fig. 1*A* except that in the final (active tension) group each dot represents 10 g. The horizontal bars below the other four myograms represent 100 msec. The left-hand pair are records from F.H.L. muscle denervated 6 days; above is the first twitch elicited after a period of rest, below the response after a period of stimulation at a rate of one every 5 sec. The middle pair are from a F.D.L. muscle denervated 17 days; above the first twitch evoked after a period of rest, below a twitch 6 sec after a 500 msec tetanus. The right hand pair are from two soleus muscles; above denervated 17 days, below 42 days.

B, graph of time to half relaxation of the twitch plotted against period of denervations. Symbols as in Fig. 1*A*; except for ▲ which represent soleus muscles in which an after-contraction prevented measurement of relaxation (hence their vertical position has no meaning).

The isometric tetanus

Genesis of tetanus in acutely denervated muscle was similar to that seen in the normal. However, the chronically denervated muscles began to show fusion at lower rates of stimulation as would be expected from their slower twitch speeds.

One difficulty was that both acutely and chronically denervated muscles showed a second increase in tension at rates of stimulation well above those

necessary for fusion. It was considered that the increase of tetanic tension at high frequencies of stimulation was due to recruitment of muscle fibres by later impulses in the tetanic train (Fig. 3). A quantitative estimate was made of this late recruitment by measuring the ratio of the maximum tetanic tension to that evoked by stimulation at a rate sufficient to give the maximum tension in normal muscles. The mean ratio was 1·11 for

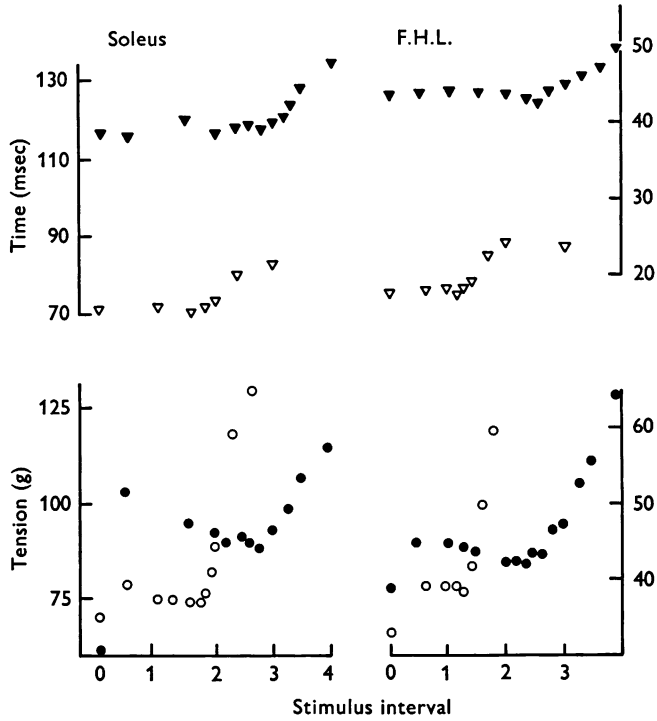

Fig. 3. The mechanical response to two equal stimuli of 0·1 msec duration applied to soleus (left) and F.H.L. muscles. In each case open symbols indicate muscles denervated for 4 days and the filled symbols muscles denervated 56 days. Stimulus interval is used as the abscissa; the active peak tension (circles, below) and the time to peak tension (triangles, above) have been plotted as ordinate. Different ordinate scales have been used for soleus and F.H.L.

soleus and 1·13 for F.H.L. In many of the records an additional contraction occurred after the tetanic train. This was probably analogous to the break contraction occurring after a very long stimulus and has been ignored in calculating tetanic tensions.

Tetanus:twitch ratio. At optimum muscle length the mean ratio of maximum active tetanic tension to peak-twitch tension in acutely denervated F.H.L. muscles was 4·71 which was 1·13 times the control mean

of 4·15. The acutely denervated solei gave a mean 1·11 times higher than the controls: 4·33 compared with 3·92. These differences could almost exactly be accounted for by the recruitment estimated above. In chronically denervated muscles the mean values were less than the controls being 2·36 for F.H.L., 2·24 for F.D.L. and 2·82 for soleus. These reductions were significant by the *t*-test at the 0·01 level in soleus. The values in the chronically denervated muscles are probably also over-estimates owing to the effect of recruitment. Details are given in Table 1.

Decay of 'active state'. A two element model of muscle suggests that, in an isometric tetanus, tension develops in a series elastic element as contractile elements shorten at a rate determined by their force:velocity relationship. In an isometric twitch the activity of the contractile element reaches its peak (not necessarily maximal) for only a brief time and then decays. At the peak of a twitch the rate of change of tension and therefore the rate of shortening of the contractile element is zero and the externally recorded tension may be equated to the intensity of 'active state' of the contractile element. The method used here was to measure the peaks of unfused tetani as well as of twitches. This has been applied to frog muscle (Edman & Grieve, 1966) and the method is illustrated in the inset of Fig. 4*A*. Each point of the graph of Fig. 4*A* is the peak tension (p) of an oscillation of tension of an unfused tetanus plotted against the rise time (t) of that oscillation. p is expressed as a fraction of P_0, the maximum tension in a fully fused tetanus. The two sets of points show results obtained from chronically denervated F.H.L. (filled circles) and soleus muscles. Double values are plotted at $t = 0$, indicating the extent of recruitment described earlier. The full curves show measurements made from several oscillations in each tetanus and from several tetani elicited at different stimulation rates.

For comparison in Fig. 4*B*, results are presented showing similar measurements made from control muscles which were curarized to allow direct stimulation. Measurements from soleus (○, ×) and for F.H.L. (●, ⊙, +) are plotted. Apart from the shorter time scale the results are qualitatively different from those of Fig. 4*A*. First, points obtained from a tetanus elicited at one frequency do not fit the curve of points obtained at a different frequency. This is particularly obvious at low stimulation rates and some of these are indicated by separate symbols (× and ○, +). Secondly, the points of Fig. 4*A* fit a straight line reasonably well, whereas those of Fig. 4*B* are best described by curves which show a change in the sign of the slope (i.e. the rise time of the response to a single stimulus in a tetanic train is maximal for the first stimulus in denervated muscle but for the second or third in the control). Regression lines fitted to the points of Fig. 4*A* have been drawn on to Fig. 4*B* to help comparison and it can be

seen that only at higher tensions (later in a tetanus of moderate or high frequency of stimulation) in soleus is there any correspondence between the control and denervated muscles.

Rates of rise. If the denervated muscles were stimulated at frequencies greater than those necessary to produce apparent tetanic fusion, the rate of development of tension also increased as it does in normal muscles (Buller & Lewis, 1965*a*). Fig. 5*A* illustrates the maximum rates found in acutely denervated (above) and chronically denervated muscles (below).

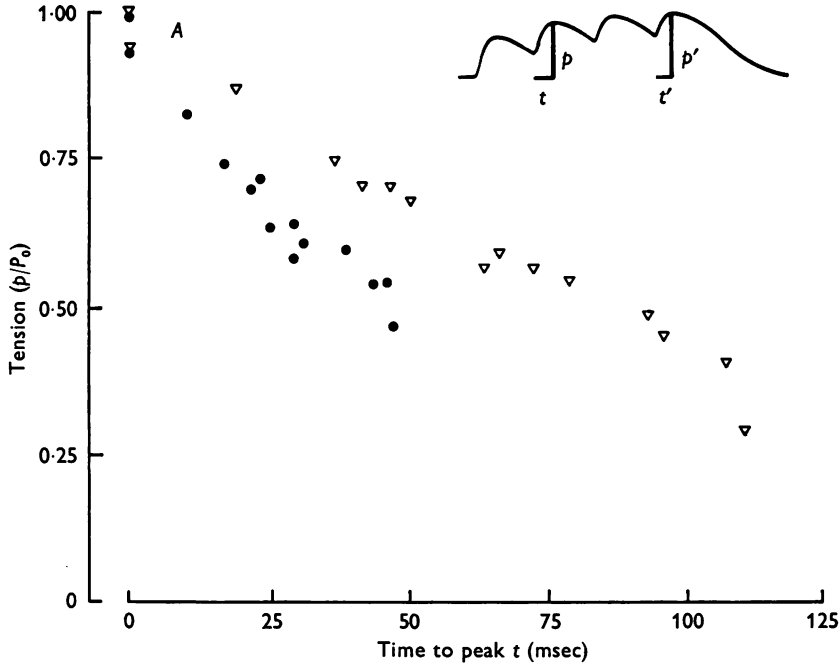

Fig. 4*A*. For legend see opposite page.

Each record consists of an isometric myogram (100 g calibration to right) shown above the rate of change of tension obtained by electrical quasi-differentiation of the tension analogue. Rate was expressed in terms of maximum tetanic tension for that muscle (i.e. as a percentage of P_0; cf. Buller & Lewis, 1965*a*). Calibration bars are displayed to the left of each differential and correspond to 1 % P_0/msec for soleus (left) and 4 % P_0/msec for F.H.L. (right). Two sweep speeds have been used, calibration bars for both columns are 0·5 sec. Although the twitches of the chronically denervated muscles were slower than those of the acutely denervated ones (see Fig. 1*A*), the tetani were comparable in their rising phases. This is shown to be true over a range of frequencies of stimulation in Fig. 5*B*. On the left results for acutely and chronically denervated soleus muscles are

compared (filled and open triangles respectively) and show only small differences. This is true of the mean values for maximum rate of rise which were 1·40 and 1·53 % P_0/msec for acutely and chronically denervated muscle respectively. The mean optimal frequencies of stimulation were also similar (200 and 195 Hz). On the right of Fig. 5*B* are similar graphs for F.H.L. Here chronically denervated muscle (open circles) showed a slower

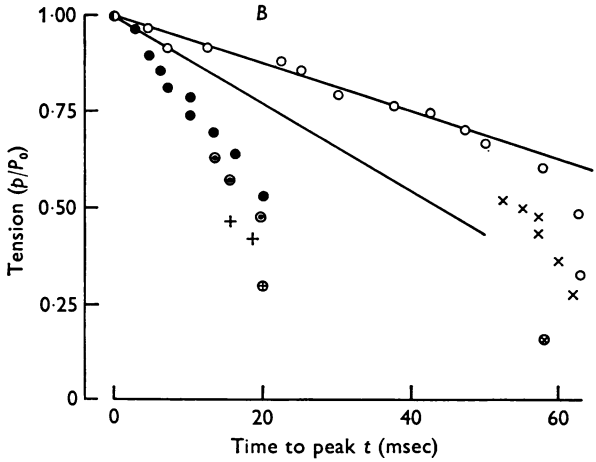

Fig. 4*A*, decay of 'active state' measured from unfused isometric tetani of F.H.L. (●) and soleus muscles (▽) denervated for 56 days. The inset myogram shows an unfused tetanus used for the measurements of the graph. The peaks of oscillation (p and p') in the myograms have been measured as a fraction of peak tetanic tension in a fully fused tetanus (P_0) and plotted as the ordinate against the time rise (t and t') of that oscillation (measured from the time tension began to increase to the time of peak tension).The measurements have been taken from several tetani elicited at different stimulation rates.

B, comparable results from directly stimulated, curarized control muscles; here symbols also indicate rates of stimulation. In soleus points indicated by × were obtained from a tetanus elicited at 10 Hz, but other points (○) were obtained at stimulation rates from 16 to 125 Hz. For F.H.L. two rates of stimulation are indicated separately (+, 25 Hz; ⊙, 32 Hz) and other rates between 40 and 164 Hz are shown as ●. The two lines are linear regression slopes fitted to the two sets of points in Fig. 4*A*.

rate of tension development (mean 3·09 % P_0/msec) than did acutely denervated muscle (mean 4·09 % P_0/msec). Also this maximum rate was reached at a lower stimulation frequency (mean 230 compared with 460 Hz). The time at which maximum rate occurred in chronically denervated muscle was 15–19 msec in F.H.L. and 19–23 msec in soleus (see Table 1).

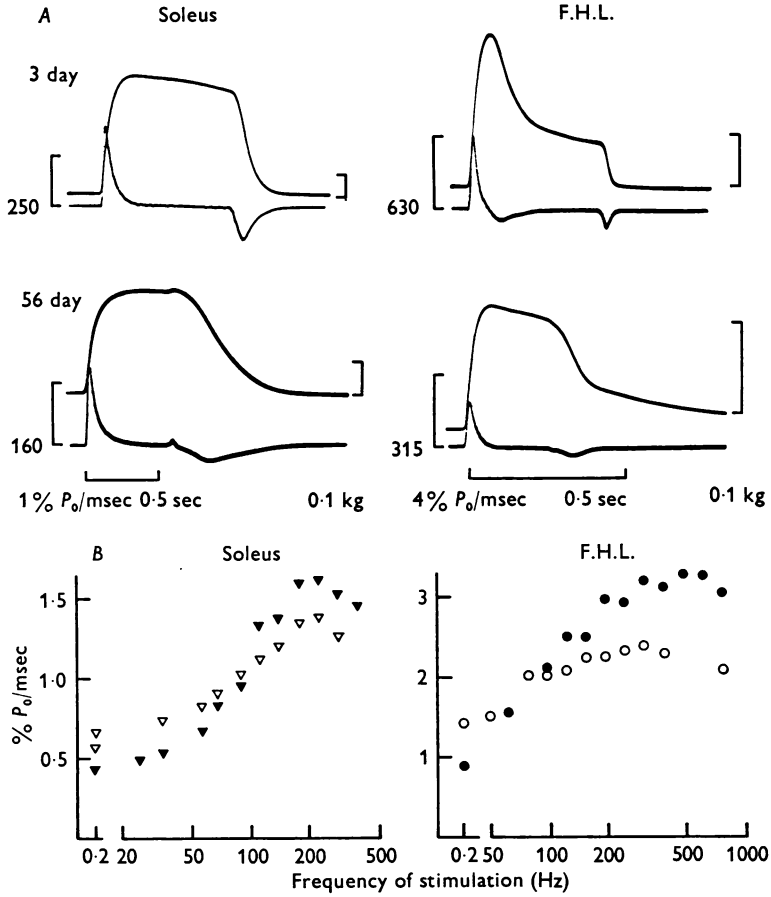

Fig. 5*A*, pairs of oscilloscope traces showing isometric tetani (above) and the rate of change of tension obtained by electrical differentiation (below). Left-hand pair are records from soleus, right-hand ones from F.H.L. muscles. The top row are muscles denervated 3 days earlier, the bottom row muscles denervated for 56 days. In each case the frequency of stimulation (shown to the left in Hz) was that which gave the greatest maximal rate of rise. The soleus muscles were recorded with a slower oscilloscope sweep and 500 msec calibration bars are shown below each column. A tension calibration representing 100 g is shown to the right of each tension record. The calibration bars for rate of change of tension are shown to the left of each trace and represent 1 % P_0/msec for soleus and 4 % P_0/msec for F.H.L. muscles.

B, plots of maximum rate of rise of tension (as % P_0/msec ordinate) against frequency of stimulation used (abscissa). A pair of soleus muscles are shown on the left, one had been denervated 3 days (▼) and the other 56 days (▽). The F.H.L. muscles (right) were from an animal operated 7 days (●) and 56 days (○) previously.

Extracellular potentials

In the muscles of four animals denervated from 24 to 46 days and 2 curarized controls electrical activity was measured with Sherringtonian electrodes on the muscle belly following a stimulus applied between the tendon and a metal twist drill in the upper tibia. An example is shown in Fig. 6*A* from a soleus muscle denervated for 24 days (left-hand column) and from F.D.L. (40-day denervation, right). Each record consists of the isometric myogram in addition to the electromyogram. In the top row the isometric myogram was recorded at high gain to show the onset of contraction and the electrical record was at low gain to record the initial compound action potential which lasted less than 5 msec. The bottom row of records were taken on a slower time base speed, with the myogram at lower gain to illustrate the shape of the whole twitch. The electrical record, now at higher gain, showed a second burst of action potentials in F.D.L. which immediately preceded the secondary contraction seen in the relaxation phase of the twitch. The time of onset varied from 60 to 100 msec after the stimulus in the four fast muscles studied. In the lower left trace of Fig. 6*A* only a few fibrillation potentials are seen and the same was true in the three other solei studied.

In Fig. 6*A* the initial compound action potential was brief, in other muscles (including curarized controls) it had a duration of 10–20 msec. In order to examine the initial response in greater detail synchronous volleys were studied by using limited stimulation of superficial bundles of muscle fibres. The tension was recorded from these fascicles and was found to have a time course similar to the twitch of the whole muscle. Fig. 6*B* shows extracellularly recorded potentials from soleus (left) and F.H.L. muscles denervated for 35 days. The top trace shows the initial response recorded with a fast sweep speed and with low gain (the calibration pulses were 50 μV in all these records). The middle two records show the after-discharge at a slower sweep speed at a low (above) and high gain. The lowest record shows spontaneous fibrillation at the same amplification for comparison. Thus an after-discharge was seen in both muscles and its time course was measured in single sweep records and those in which six oscilloscope sweeps were superimposed. In F.H.L. the after-discharge occurred between 105 and 135 msec after the stimulus. In soleus some activity occurred at 90 msec but it was most intense between 140 and 175 msec. Increased activity persisted some ten to twenty seconds after the stimulus.

Conduction velocity. In all traces of Fig. 6*B* the recording electrode was held in one position. It was possible to measure conduction velocity by moving the recording electrode in steps from the stimulating electrode.

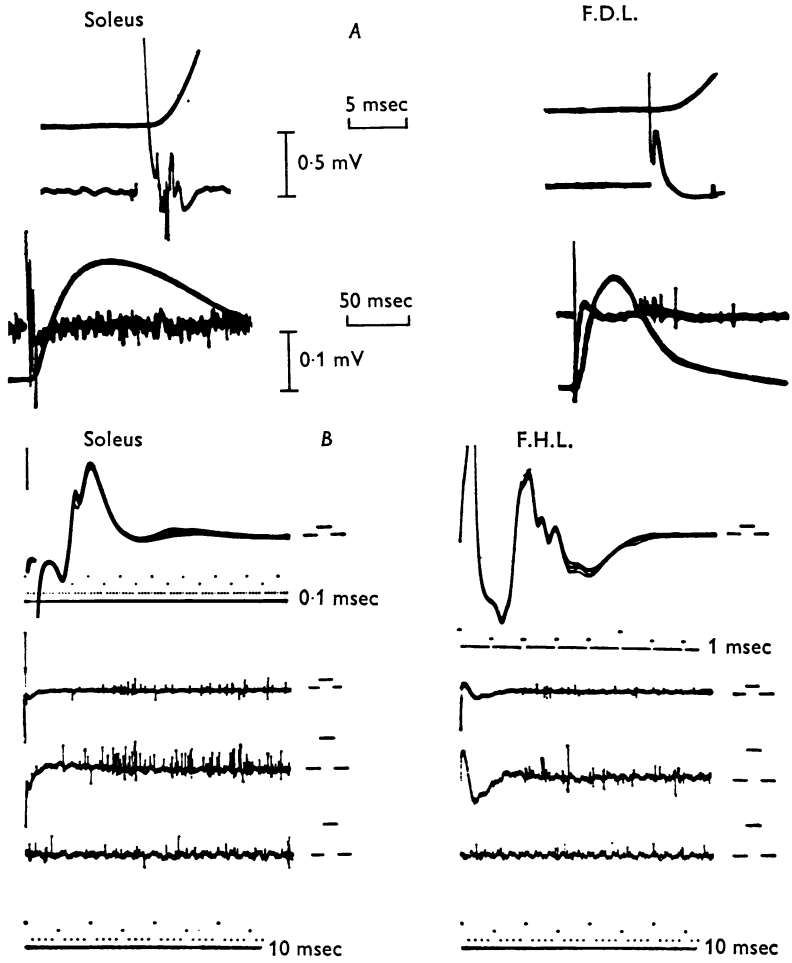

Fig. 6*A*, potentials recorded with a concentric needle electrode from denervated muscles; soleus (left, denervated 24 days, stimulus 1 msec and 40 V), and F.D.L. (right, denervated 40 days, stimulus 0·3 msec, 100 V). Isometric tension is also recorded. The traces in the top row were recorded at a fast sweep speed and the tension is the upper beam. In the lower row a slower sweep was used to show late electrical changes (upper beam) at a higher amplification, tension was recorded at lower gain to show the full time course of the twitch. Calibration bars for sweep speed and e.m.g. amplitude are shown for each row.

B, potentials recorded with bipolar needle electrode from a bundle of muscle fibres stimulated locally from a concentric needle electrode positioned about 3 mm away, left soleus and right F.H.L., both denervated for 35 days. The top traces in both columns show the compound action potential at low gain (five responses superimposed). They were recorded with a fast sweep, each with a time marker on the lower beam. The remaining three traces were recorded with a slower sweep (see time traces at foot of columns) and are single responses. The first of these three is recorded at the same gain as the top record. Below the response was recorded at higher gain and finally at this gain a trace showing spontaneous fibrillation. Calibration pulses to the right of each trace are 50 μV. Some of the spikes have been retouched.

Measurements of latency (made to the peak of the potentials in order to give some estimate of a mean value) were plotted against interelectrode distance to give a reliable estimate of conduction velocity. In a few muscles the times to the beginning and end of the compound action potential were measured. The two regression lines diverged, indicating that not all the fibres in the bundle had the same conduction velocity. In none of the experiments was there any evidence that the initial spike in denervated muscle was less synchronous than that in the controls.

Conduction velocity was measured in two or three bundles of fibres in each muscle of four cats operated from 36 to 56 days earlier. In soleus the mean value was 2·28 m/sec compared with a normal mean of 2·95 m/sec. The change was greater in F.H.L., the control muscles having a mean of 3·66 m/sec and the denervated 1·86 m/sec (see Table 1).

It was of interest to compare the effects of the conduction velocity change on the total conduction time along the muscle bundles. The mean time for control soleus was 12 msec (range 10–16) compared with 15 msec (10–20) for denervated soleus. F.H.L. changed from 7·5 msec (7–8) to 13 msec (7–17). These figures are high as the bundles chosen for measurement were the longest in each muscle.

One F.H.L. was recorded 4 days after denervation. Its conduction velocity of 2·90 m/sec was just within the normal range.

Intracellular recording

In order to study responses to single stimuli in individual fibres intracellular records were made in two cats denervated for 35 and 37 days. F.D.L. was examined in one animal, F.H.L. in another and soleus in both. Measurements of the membrane potential were made and the action potential recorded on a fast oscilloscope sweep in a number of fibres. Fig. 7 shows records obtained from control muscles (above) and muscles denervated for 37 days (lower row). The left-hand column shows soleus muscle fibres and on the right are F.D.L. fibres. Each record consisted of two superimposed sweeps, one with and one without the stimulus. An interval of 6 sec occurred between the two and only those fibres which showed a deterioration of less than 5 mV in the resting potential were used to measure the action potential.

A. J. Buller, D. M. Lewis & R. M. A. P. Ridge (unpublished observations) have found significant differences between the membrane potentials of normal slow and fast muscle in the cat (mean values were 80·1 and 85·1 mV respectively). These differences were lost in muscles denervated for 37 days and the potentials were smaller. The mean resting potential in soleus was 67·6 mV (S.E. of mean = 1·4, n = 29) and F.D.L. 65·3 mV (S.E. of mean = 1·0, n = 31). The mean amplitude of the action potential in soleus was 74·1 mV (S.E. of mean = 2·8) and in F.D.L. 72·2 mV (S.E. of mean = 1·5); again these differences were not significant. Thus action potentials

did not show reverse polarity in all fibres. A positive after-potential was often seen and had a mean value of 69·5 mV (S.E. of mean = 1·5, n = 18) in soleus and 75·8 mV (S.E. of mean = 1·7, n = 19) in F.D.L. This may be contrasted with a negative after-potential seen in normal cat muscles.

The rise time of the action potentials was longer than normal (Buller *et al.* 1965), the distribution of these values was skewed towards long values in F.D.L. and was bimodal in soleus. The longest rise times may have been obtained from more damaged fibres and the modal values may best represent the rise time of denervated fibres and these were 330 μsec in soleus and 350 μsec in F.D.L. The time to fall to one third of the peak value was also measured and had a mean value of 1·01 msec (S.E. of mean = 0·04, n = 25) in soleus and 1·16 msec (S.E. of mean = 0·03, n = 27) in F.D.L. fibres.

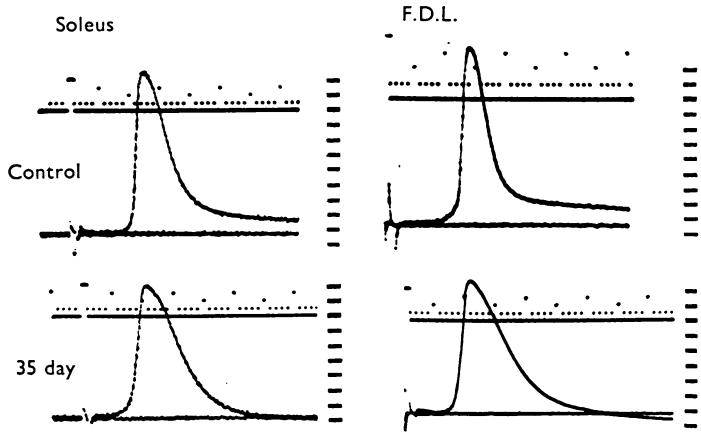

Fig. 7. Intracellular records from soleus (left) and F.D.L. muscle fibres. The bottom row are from fibres of muscles denervated for 37 days, the upper records are controls of the opposite limb. The 0·1 ms time markers also indicate zero volts and the calibration bars are at 10 mV. The largest pulse on the time trace indicates the time of the stimulus. Each intracellular record consists of two superimposed traces, one with and one without a stimulus; the time between sweeps was 6 sec. Some of the traces have been retouched.

Thus the resting and action potentials were smaller than normal, and the total duration of the action potential was prolonged in both muscles. All the changes were more extensive in the fast muscle and none of the means obtained in denervated F.D.L. were significantly different from those measured in denervated soleus.

By using a somewhat less demanding criterion of the stability of a penetration, a larger number of fibres could be examined for a repetitive response to a stimulus. All the fibres responding (132 fibres in soleus and 89 in fast muscles) showed only one action potential within 5 msec of the stimulus. A number of these fibres were also recorded on an oscilloscope sweep with a duration of 0·5 sec. Examples are shown in Fig. 8*A* from a soleus (left) and F.H.L.(right) both denervated 35 days. Two types of

electrical activity were seen in single fibres and have been selected to illustrate Fig. 8 although both types of response were seen in all muscles. Some fibres showed only a single spike in response to a stimulus and this type was seen in a minority of fibres examined in fast muscles (39 % in the F.D.L. and 37 % in F.H.L.). It was more common in soleus although the proportion was very different in the two muscles examined (41 and 85 %). In other fibres the initial spike was followed by a repetitive discharge at about 10 Hz and the latency of the second spike from the

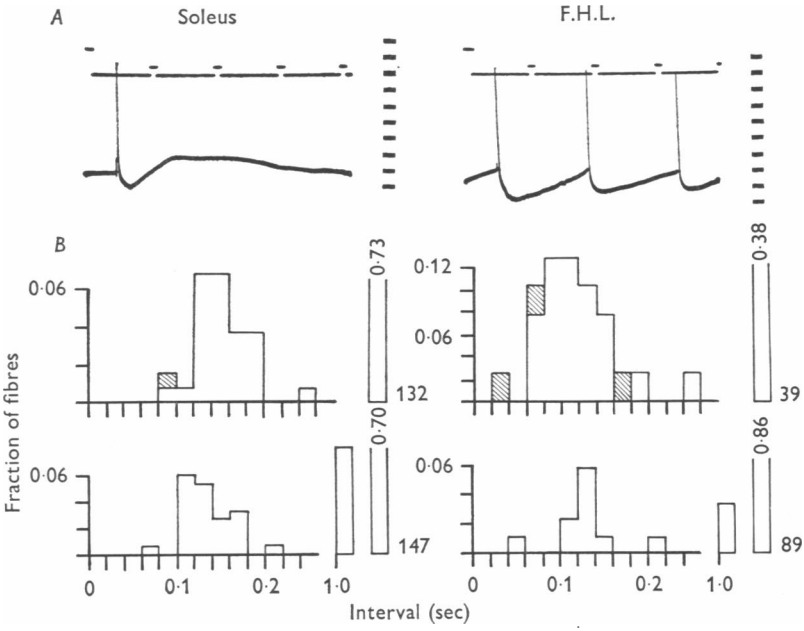

Fig. 8. *A*, intracellular records from soleus (left) and F.H.L. muscles denervated for 35 days. The responses have been selected to show two types of fibre (both of which are seen in both muscles). Time markers are at 100 msec. Voltages are indicated as in Fig. 7. Stimuli are delayed 50 msec after the start of the sweep. Spikes have been retouched.

B, histograms of repetitive activity seen in intracellular recording from single fibres in denervated muscle. Intervals are counted in 20 msec bins and the lower class limits are shown at the foot of the Figure. The frequency of occurrence of each interval is shown by the ordinate scale or by a figure at the top of a column if the scale is exceeded. The number of fibres studied is shown on the far right. The top pair of histograms show the time of occurrence of a second spike following a single stimulus. The far right bins show fibres in which no second spike was seen, and the shaded areas indicate spikes occurring during loss of the fibre accompanied by sudden fall of potential. The top right histogram was obtained from an F.H.L. muscle denervated 35 days, the top left from two solei denervated 35 and 37 days. The lower pair of histograms were from the same muscles and a F.D.L. denervated 37 days, and indicate the mean interval of spontaneous fibrillation of fibres. The far right bins show those fibres without fibrillation.

stimulus is shown by the top pair of histograms of Fig. 8*B* (soleus left). The shaded areas indicate action potentials which occurred during a sudden loss of the membrane potential and were considered to be due to mechanical injury to the fibre during contraction. Similar effects were seen in fibres which gave no initial response to the stimulus. These injury spikes were excluded in calculating that the mean time to the second action potential was 165 msec (S.E. of mean = 9, n = 29) in soleus and 108 msec (S.E. of mean = 9, n = 24) in the fast muscles.

In total, 187 fibres from soleus and 154 from fast muscles were penetrated and in the absence of stimulation, action potentials were seen in a proportion of these. In some the discharge persisted for less than 6 sec and was considered to be due to injury, as were spikes in others which occurred only in phase with respiratory movement. If these were excluded, between 8 and 20% of fibres showed continuous activity which was considered to be spontaneous fibrillation. The lower pair of histograms of Fig. 8*B* show the mean intervals observed in fibres in which fibrillation was sufficiently stable to make measurements. The mean value in soleus was 121 msec (S.E. of mean = 5, n = 15) and in F.H.L. 124 msec (S.E. of mean = 9, n = 10).

DISCUSSION

In the chronically denervated muscle the most prominent effect was the slowing of twitches, especially marked in the relaxation phase. The change was greatest in the fastest muscle (F.H.L.) but also occurred in soleus which contrasts with the results reported by Eccles *et al.* (1962). Change occurred abruptly in the third week of denervation and perhaps Eccles *et al.* missed this later phase. Possibly a transition stage was represented by the few muscles in the second week that showed a reversible increase of twitch speed.

All the measurements of electrical activity agree that the initial response of denervated muscle to a stimulus is a single action potential and it may be concluded that the resulting contraction is a true twitch and may be compared directly with the response of normally innervated muscle. A repetitive after-discharge has been seen and it can be correlated with the after-contraction seen in the mechanical response. Clearly in fast muscle the time of occurrence of the second spike of the repetitive response at about 100 msec corresponds to a late phase of relaxation in the twitch and just precedes the time of onset of the after-contraction (110 msec). In soleus the after-discharge was maximal at about 150 msec and the after-contraction began at 180 msec. However this reactivation occurs relatively earlier in the relaxation phase and may be expected to produce slowing of relaxation and an over-estimate of time to half relaxation in

those muscles in which it was present. Soleus less commonly showed a repetitive response, so the error in the mean value of half relaxation time will be smaller than would be expected from observations on muscles which do show this response.

Chronically denervated muscle had a low conduction velocity and this would slow the twitch. The maximum effect would be in F.H.L. in which the velocity fell from 3·8 to 1·9 m/sec. The longest fibres are 25 mm (W. S. Al-Amood & R. Pope, personal communication) and this change of conduction velocity could account for an increase of about 7 msec in the time to peak.

The slow conduction velocity might be expected to have a greater effect on the rate of change of tension in a tetanus which reaches a peak in a time about one third of the time to peak tension in a twitch, comparable in F.H.L. with maximum muscle fibre conduction times. An estimate of the effect may be based on assumptions that sarcomeres are activated in series and that each develops tension with an identical time course that may be represented as $f(t)$ where t is time. The recorded tension at a particular time T will be given by

$$\frac{1}{C}\int_{T-C}^{T} f(t)$$

where C is the total conduction time along muscle fibres. This time will depend not only on conduction velocity but also on conduction distance. Motor end-plates are found in the middle third of the muscle but directly stimulated fibres may be activated at one end nearest the cathode and so will have longer conduction distances.

Tetani of normal muscles reach a maximum rate of rise of tension of 4·64% P_0/msec at a time of about 8 msec (Buller & Lewis, 1965*a*). Numerical integration of tension differential curves allows the prediction that if conduction time (C) were increased by 100% (doubling conduction distance) the maximum rate of rise of tension would fall to 4·1% P_0/msec and occur at 12 msec. If conduction velocity were then halved these values would change further to 3·0% P_0/msec and 17 msec. These changes are of the same order of magnitude as those seen in acutely and chronically denervated F.H.L. respectively. In soleus the effect would be smaller since conduction times were shorter relative to the time to maximum rate of rise of tension. In normal soleus the peak rate was 1·53% P_0/msec occurring at about 18 msec. Doubling conduction distance is predicted to increase time to peak rate to 22 msec and decrease the maximum value to 1·4% P_0/msec. The decrease of conduction velocity in chronically denervated muscle was 23% and this would change the two parameters to 25 msec and 1·3% P_0/msec. Again these are comparable with observed values.

Thus it may be argued that the changes of denervation may not include a fundamental change in the rate at which tension develops in the sarcomere. From this it might be predicted that there would be no change in rate of isotonic shortening (cf. Close, 1964). This was suggested for the rabbit by Roberts (1916). Elliott & Thomson (1963) claim to have shown a change in the rat but without measurement of isometric tetanic tension their results must necessarily be interpreted with caution.

If, however, the changes in the rate of rise of tetanic tension are taken as estimates of changes occurring at the sarcomere level certain discrepancies still remain. The observed reduction of rate of change of tension in chronically denervated F.H.L. was 33% from normal values and 25% from that measured in acutely denervated muscle. Close (1965) has shown that the maximum rate of isotonic shortening per sarcomere is inversely related to twitch contraction time. Buller & Lewis (1965*b*) have demonstrated a similar relationship between maximum rate of rise of tension in a tetanus and the time to peak of a twitch. If this were to hold true in denervated F.H.L. a reduction of the rate of change of tension by 24 or 33% would be associated with an increase in the time to peak of the twitch to 24 or 29 msec. The observed increase to 41 msec suggests that this relationship cannot hold. The discrepancy is also seen in chronically denervated soleus where the maximum rate of rise was almost normal but the time to peak of the twitch was prolonged by 51%.

The reduction of tetanus–twitch ratio can be related to these disproportionate increases in time to peak. One possible explanation is that more complete activation of the contractile proteins of denervated muscle occurs in response to a single action potential. There is indirect evidence that activation is incomplete in normal mammalian muscle. As in Fig. 4*B* attempts to trace the decay of 'active state' using measurements of unfused tetani in normal cat muscle result in complex curves which vary with frequency of stimulation (A. J. Buller & D. M. Lewis, unpublished observations; Ranatunga, 1969). It may be suggested that the complexity is due to increasing activation with each stimulus of the tetanic train. In contrast the curves shown in Fig. 4*A* are as simple as those obtained in frog muscle (Edman & Grieve, 1966; Ranatunga, 1969). Current theories of contraction suggest that the shape of isometric tetanic tension curves in the sarcomere is determined mainly by the rate at which linkages are formed (Huxley & Simmons, 1971). If the rate of formation of linkages were itself related to tension, as it is in an isotonic contraction, the peak of a twitch would still have a special significance in relation to activation within a fibre. Moreover, the qualitative differences between curves of 'active state' decay in amphibian muscle and those obtained in the mammal and the change following denervation suggest that some fundamental differences underlie them.

The over-all durations of the action potential in fast and slow muscle were more than 50% greater than the values obtained in normal muscle and differences between fast and slow muscle were lost. It is unlikely that this slowing resulted from injury to the smaller fibres of denervated muscle since it has been observed in normal muscle that although injury (as evidenced by a low and deteriorating membrane potential) slows the rising phase of the action potential it has little effect on the over-all duration. In addition an increase in refractory period has been observed to follow denervation and this is in line with the prolongation of the action potential. The long duration of the action potential offers a mechanism by which the twitch response might become slow for, although the muscle action potential is small, the period of critical depolarization would be longer. However, other possible mechanisms exist: for example the diameter of fibres is reduced (Tower, 1935) so that the inward spread of activation could be more extensive (Adrian, Costantin & Peachey, 1969; but contrast Gonzales-Seratos, 1971). Again the atrophy of denervation involves the contractile protein more than sarcoplasmic reticulum (Pellegrino & Franzini, 1963) which could increase and prolong activation. The apparently abrupt transition from muscle with normal contraction characteristics to the chronically denervated type of behaviour does require explanation and is not related to the atrophy which has been described as progressive.

In summary, the changes in chronically denervated soleus may be explained by an increase and prolongation of activation following a single stimulus. Similar changes have been found in chronically denervated F.H.L. but are accompanied by some slowing of the maximum rate of rise of tension. The changes in twitches and tetani were greater in fast muscle but the contractions of F.H.L. remained distinct from those of soleus.

Eccles *et al.* (1962) have compared the increase in time to peak of twitches of F.H.L. after denervation with that following cross-innervation. Measurements of tetani reported here indicate that the mechanisms of the changes in the two cases are different and that the similarity between the twitches is fortuitous. The slowing of twitches which occurred in both fast and slow muscle after denervation is probably more a consequence of the type of fibre atrophy. Despite the absence of neural influence fast and slow muscle retain their separate identities and any theory of the mode of action of motor nerves must account for this. Buller *et al.* (1960) showed that soleus became faster than normal following spinal transection and discussed, among others, the possibility that this was caused by the absence of a neurotrophic factor that normally makes this muscle slow. The present experiments in no way invalidate the possibility of trophic factors

but they do suggest that the observations of Buller *et al.* cannot be explained simply by the absence of a specific factor.

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